Benha Veterinary Medical Journal 45 (2023) 77-81

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Biochemical and histopathological changes of L-arginine experimentally induced acute pancreatitis in rats

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ARTICLE INFO

Keywords

L-arginine, Acute pancreatitis, Exocrine pancreatic enzymes, Proinflammatory cytokines

Received 25/11/2023
Accepted 13/12 /2023
Available On-Line 31/12/2023

ABSTRACT

The biochemical and histopathological changes of L-arginine experimentally induced acute pancreatitis (AP) in male rats were studied. Forty rats were separated into two groups (20 rats each). control: rats were fed normal diet. Acute pancreatitis group (AP): AP was induced by injecting 300 mg/kg bwt of L –Arginine (Arg) intraperitoneally. Blood samples were collected after 6, 24 and 96 hours and 7 days. The separated serum samples were directly used for the estimation of amylase, lipase, ALT and AST activities besides glucose levels. Also, TNF-α, IL-6, NO, total cholesterol, triglyceride, urea and creatinine levels were determined. The results revealed substantial increases in serum amylase, lipase, ALT and AST values as well as TNF-α, IL-6. NO, serum urea and creatinine levels. Meanwhile, non-significant changes in plasma, glucose and serum triglycerides levels were observed, while cholesterol showed non-significant decreased specially at 6 and 24hrs and significant elevation at 96hr. post-induction of AP. Histopathological changes of L-arginine treated group showed, focal degeneration and necrosis of the pancreatic acini at 24 hours post- induction of AP, while at 7th day focal area of acinar epithelium regenerations was recorded.

1. INTRODUCTION

AP is a potentially fatal inflammatory illness with serious consequences for the patient's health (Kumar and Robbins 2007; Melo et al., 2010). Although the pathogenesis of acute pancreatitis is not fully understood, oxidative stress, leukocyte activation, and microcirculatory disturbances are the main events that are characterized by generalized inflammatory cell infiltration, activation of digestive proteases, and the release of different types of inflammatory mediators such as IL-6 as predictors of severe AP (peter et al.,2021). One of the most significant mechanisms of cell damage is oxidative stress, which occurs when damaged pancreatic acinar cells and activated immune cells produce high amounts of ROS, causing an imbalance in the oxidative stress/antioxidant balance (El-Ashmawy et al., 2018).Abdel-Aziz et al., (2020)stated that treated rats with a single daily either 100 or 300 mg/kg/day of L -arginine orally for 14 successive days, showed pancreatic microscopic lesions with elevated serum amylase enzyme levels. Additionally, AP rats showed increased pancreatic TNF-αlevels with decreased IL-10 levels. Furthermore, MDA and NO were elevated in AP, whereas SOD activity and GSH levels were lowered. In addition, Fawzy et al., (2021) discovered that L-arginine promoted AP in rats via increasing serum amylase and lipase levels. The ALT and AST levels were strongly connected with the severity of pancreatitis, and they return to normal when pancreatitis is resolved (Kanwal et al., 2021). Acute kidney damage is a common severe consequence of severe acute pancreatitis (SAP) and a significant predictor of morbidity and death in critically sick septic individuals. The goals of this study were to look into the serum biochemical changes and histological changes in L-arginine-induced acute pancreatitis in male albino rats.

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2. MATERIAL AND METHODS

2.1. Animals and chemicals

The current study included 40 male rats weighing between 220 and 250 g. They were taken from Benha University's Experimental Animal Centre, Faculty of Veterinary Medicine. The rats were kept separately in a metal cage with unrestricted access to water. Throughout the trial, rats were kept under stable and nutritious environmental conditions. Rats were acclimatized for 15 days before starting the trial.
L-arginine of the highest purity (essential amino acid) reagent grade, ≥ 98% (TLC) powder form was obtained from Sigma-Aldrich CO.st Louis, USA.

2.3. Induction of acute pancreatitis

L-arginine solution was prepared at a concentration of 10 % with saline in sterile tubes with screw caps. The fresh solution was prepared just before injection. Animals were accurately weighted and the volume of

10% L-arginine solution was calculated and injected with 300 mg/kg body weight intraperitoneal (I.P)for induction of acute pancreatitis and the equivalent volume of normal saline was injected in the control group.

2.4. Experimental design

Forty albino rats were separated into 2 groups as follows:
Group (1) (control): contains 20 rats given sterile saline by i.p injection.
Group (2) (AP induction): contains 20 rats were injected i.p with a single dose of 10% L-arginine (300 mg/kg body weight) according to the modified method (Soliman et al., 2019).

2.5. Sampling
2.5.1. Blood samples

Blood samples were collected from the retro-orbital venous plexus at 6, 24, 96hr. and 7 days using dry, clean, screw-capped tubes. By centrifugation for 15 minutes at 2500 r.p.m, serum was separated. The sera were stored at -20°C.

2.5.2. Tissue samples

Tissue samples were collected from the pancreas of each rat in diverse groups at 6, 24,96 hr. and 7th day every checkpoint of the experiment and preserved in neutral buffered formalin (10 %) for histopathological evaluation.

2.6. Biochemical analysis

Serum Amylase was estimated by enzymatic colorimetric method (Winn-Deen et al.,1988),serum lipase level was evaluated using lipase ELISA kit (Elabscience Biotechnology Inc, USA, Catalog No: E-EL-R2466) according to the manufacturer instructions,Interleukin-6 (IL-6) by ELIZA technique (Chan and Perlstein 1987), using kits from My Bio source company and Tumor Necrotic Factor--α (TNF-α)by ELIZA technique for quantitative determination according to (He and Ting 2002) from ALPCO Diagnostics company , and the glucose concentration in the sample was estimated according to the method of (Trinder ,1969), Nitric oxide (NO) activity was measured as the method reported by (Montgomery and Doymock 1961). ALT was estimated according to (Murray, 1984a). AST was estimated according to the method of (Murray, 1984b). Serum cholesterol was determined by enzymatic colorimetric (Allain et al., 1974). Colorimetric determination of triglycerides was carried out according to (Fredrickson et al., 1967) , urea was estimated according to (Kaplan, 1984) and creatinine was estimated according to the method (Henry, 1974).

2.7. Histopathological Examination

Small tissue specimens were collected from the pancreas of rats in all groups. These specimens were fixed in 10% neutral buffered formalin. Following proper fixation, specimens were dehydrated in ascending grades of ethyl alcohol before being cleaned in xylene and embedded in paraffin wax about 5µm thick tissue paraffin sections were cut using a rotatory microtone and hematoxylin and eosin stain were used for staining of these sections according to Bancroft et al (1996)

2.8. Statistical analysis

Data were statistically analyzed using one-way ANOVA with a post hoc Duncan multiple comparison test using SPSS for Windows version 20. At a P value of 0.05, differences were judged significant.

3. RESULTS

3.1. Serum Amylase, Lipase and Glucose level changes in AP
Data demonstrating the effects of L-arginine induced pancreatitis on serum amylase, lipase and glucose after 6, 24, 96 hours and 7th day of injection were summarized in Table (1), serum amylase and lipase activities showed significant increases in L-arginine group compared with control. The values were increased starting from 6hr. post inoculation and gradually elevated and the peak of the level was reached after 24 hr. post inoculation. Then the level started to decline but was still over the level of the control group till the 7th day post-inoculation. On the other hand serum glucose level showed non-significant alterations in tested group in the comparison with control.

Table (1): Serum amylase, lipase and glucose level in L-arginine induced AP in comparison with control group.

Period	Amylase (U/l)	Lipase (U/l)	Glucose (mg/dL)
0 (control)	5331.68±1101.84^{a}	14.0±0.57a	111.26±5.77^{a}
6 hr.	18122.4±2135.75^{cd}	32.2±0.52^{d}	106.21±5.95^{a}
24 hr.	23559.12±1130.13^{e}	27.16±0.45^{bcd}	113.12±6.22^{a}
96 hr.	18122.4±1654.34^{cd}	22.68±0.70^{bc}	109.23±8.12^{a}
7th day	16914±1812.24^{c}	15.86±0.50^{a}	112.52±7.72^{a}

a, b,c and d: There is no significant difference (P>0.05) between any two means, within the same column that have the same superscript letter

3.2. Serum urea and creatinine levels in AP
Serum urea and creatinine levels were significantly elevated after induction of acute pancreatitis by L-arginine at 96hr. compared with control one (Table 2).

Table (2): Urea and Creatinine level changes in L-arginine induced AP in compared with control group.

Period	Urea (mg/dL)	Creatinine (mg/dL)
0 (control)	35.83±3.81^{a}	0.82±0.07^{a}
6 hr.	44.83±8.01^{ab}	0.71±0.06^{a}
24 hr.	39.45±3.12^{a}	0.73±0.04^{a}
96 hr.	63.85±14.55^{b}	0.94±0.07^{b}
7thday	35.65±2.96^{a}	0.68±0.03^{a}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column that have the same superscript letter

3.3. Serum Pro-inflammatory changes in AP

Regarding serum pro-inflammatory results, there were significant increases in TNF-α and IL6 levels and the highest increase was recorded at 6 hours in L-arginine treated group compared to control group (Table 3).

3.4. Nitric Oxide (NO) levels in AP

Serum nitric oxide levels in L-arginine group were significantly increased at 6, 24 hours compared to control group (Table 3).

Table (3): TNF-α and IL-6 and NO level changes in L-arginine induced AP in compared with control group.

Period	TNF-α (pg/ml)	IL-6 (pg/ml)	Nitric oxide (µmol/L)
0 (control)	8.55±3.22^{a}	41.88±10.17^{a}	2.62±0.73^{a}
6 hr.	73.11±7.6^{d}	340.20±33.06^{b}	11.23±2.92^{b}
24 hr.	51.46±7.38^{c}	242.93±30.72^{c}	15.50±1.77^{c}
96 hr.	42.60±4.42^{bc}	150.51±12.88^{b}	2.62±0.59^{a}
7th day	29.80±7.61^{b}	120.20±34.74^{b}	2.48±0.98^{a}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column that have the same superscript letter.

3.5. Liver parameters and Lipid profile in AP

Data showing the effect of one dose L-arginine injection on ALT, AST, cholesterol and triglyceride were summarized in (table 4). There was a significant increase in activates of ALT of tested group compared with control group during check points at 6, 24 hours, while non- significant increased at 96 hours and 7thday. Also, AST activities were significantly increased in all check points in comparison with control one. Concerning serum lipid profile, cholesterol levels at 6, 24hrs. were non-significantly decreased but at 96hr and 7thdays there were significant changes compared to control while triglycerides levels at same intervals were recorded non-significant changes.

Table (4): Serum ALT, AST, and lipid profile changes in L-arginine induced AP in rats

Period	ALT (U/L)	AST (U/L)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
0 (control)	109.48 ±7.05^{a}	181.82± 12.64^{a}	66.66± 5.30^{ab}	132.99± 10.02^{a}
6 hr.	204.67 ±25.37^{b}	357.62± 25.72^{b}	64.47± 3.27^{ab}	124.60± 3.73^{a}
24 hr.	199.45± 12.87^{b}	350.88± 33.60^{b}	61.90± 4.91^{ab}	134.43± 15.51^{a}
96 hr.	125.28± 16.00^{a}	333.36± 23.26^{b}	71.14± 5.51^{b}	149.00± 9.5^{a}
7th day	124.25± 21.43^{a}	280.86± 42.77^{b}	57.99± 3.44^{a}	151.53± 9.24^{a}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column that have the same superscript letter.

3.6. Histopathology

The microscopic examination of the pancreas sections in AP induced by L–arginine showed focal acinar necrosis characterized by pyknosis of nuclei and deeply stained cytoplasm 24 hours post induction of AP (Fig.1) Also, some examined pancreas of rat showed moderate vacuolar and hydropic degeneration of some acinar cells was also seen (Fig. 2). At 96 hours post induction of AP. The examined pancreas section showed mild periductal fibrosis and infiltrated with inflammatory cells (Fig. 3) showed with focal area of lytic necrosis with marked vacuolar and hydropic degeneration of surrounding acinar cells (Fig. 4). At 7th day post induction of AP, focal hyperplasia of pancreatic ductal epithelium (Fig. 5) with focal area of acinar epithelium regeneration characterized by enlarged vesiculate nuclei with pale basophilic cytoplasm were observed (Fig. 6), while the examined pancreas in control group displayed normal parenchyma (Fig. 7).

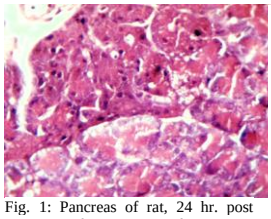

Fig. 1: Pancreas of rat, 24 hr. post induction of AP, showing focal acinar necrosis characterized by pyknosis of nuclei and deeply stained cytoplasm. H&E stain x 400.

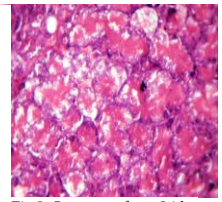

Fig.2: Pancreas of rat, 24 hr. post induction of AP, showing moderate vacuolar and hydropic degeneration of some acinar cells. H&E stain x 400.

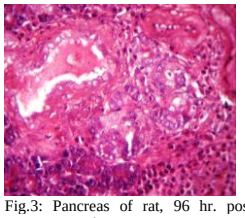

Fig.3: Pancreas of rat, 96 hr. post induction of AP, showing mild periductal fibrosis and infiltrated with inflammatory cells. H&E stain x 400

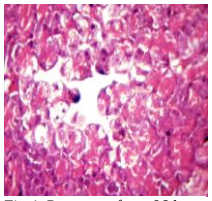

Fig.4: Pancreas of rat, 96 hr. post induction of AP, showing focal area of lytic necrosis with marked vacuolar and hydropic degeneration of the pancreatic acinar cells. H&E stain x 400

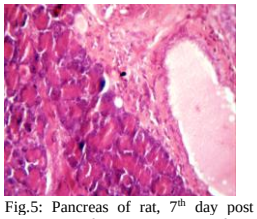

Fig.5: Pancreas of rat, 7th day post induction of AP, showing focal hyperplasia of pancreatic duct epithelium. H&E stain x 400.

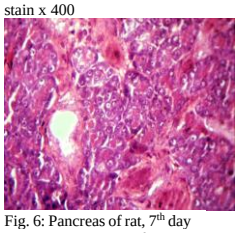

Fig. 6: Pancreas of rat, 7th day post induction of AP, showing focal area of acinar epithelium regeneration characterized by enlarged vesiculate nuclei with pale basophilic cytoplasm. H&E stain x 400.

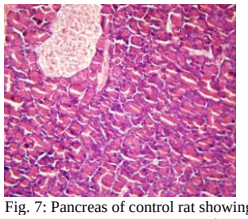

Fig. 7: Pancreas of control rat showing normal histological structures of acini and island of Langerhans. H&E stain x 200.

4. DISCUSSION

The benefits of the single dose of L-arginine induced model include its high reproducibility and ability to achieve selective dose-dependent pancreatic acinar cell necrosis, as well as its ease of adaptation and ability to titrate the severity of the induced pancreatitis down from a very severe pancreatic

necrosis and pancreatitis. So it a useful means for studying the development and recovery from pancreatic necrosis (Chan and Leung 2007). Also, this model may be useful for assessing extra-pancreatic organ damage and its oxidative stress pathways. The negative effect caused by free radicals in the body is regarded to play a key role in aging and illness, and it may induce liver injury; mechanisms of liver injury induced by TNF-α include direct hepatotoxicity and Endotoxin-induced apoptosis of hepatocytes and Kupffer cells prior to cell damage (Abdel-Aziz et al., 2020).Our experimental results were demonstrated in table (1) the effect of single dose of L-arginine induced pancreatitis on serum biochemical parameters after checkpoints6, 24, 96hrs. and 7th day of injection. A significant increase in serum amylase and lipase were recorded in the comparison with the control one. This could be attributed to imbalance between oxidant and antioxidant system with damage in acute pancreatitis (AP) experimental model. When activated leucocytes elevated the ROS production in AP, Oxygen Free Radicals (OFR) attack lipids and proteins in biofilms and cause lipid oxidation in cell membrane, protein cell solute, DNA and macromolecules in nucleus. Furthermore, with increase the production of ROS in acute pancreatitis, the intrinsic defense mechanism leads to damage of cell membrane. The pathological alteration was clear in the present with study where the examined pancreas of AP(Fig.1) showed focal acinar necrosis after 24 and 96 hrs. post-induction. These results were agreed with (Özgül et al., 2019). Observed typical acinar damage and inflammation were confirmed by a serum amylase level elevation (Amy et al., 2014; Ali et al.,2020). Fawzy et al., 2021 reported that acute pancreatitis showed inflammation with lobular atrophy, apoptosis and vacuolar degeneration of acinar cells. Non- significant differences in the serum glucose levels were detected in the tested groups in the comparison with the control group due to using of only a single dose of L-arginine that induced mild acute pancreatitis without B cell injury. This result came paralleled with (Abdel-Aziz et al.,2020) who found that blood glucose levels were unaffected in all induced acute pancreatitis groups, which contradicts another study of acute pancreatitis in rats, which induced AP by repeated doses of L-arginine, causing cell injury and affecting endocrine function of the pancreas (Abd El-Rahman,2019).

Our experiment reported the effect of L-arginine-induced acute pancreatitis on urea and creatinine values at check point 96-hour group showed significant elevation compared with control one, Acute Kidney Injury (AKI) associated with AP could be attributed to hypoxemia, the release of pancreatic amylase from the damaged pancreas, and disruption of renal microcirculation. These results agree with (Amy et al., 2014 and Mayumi et al., 2002). The severity of AP was associated with higher incidence of Acute Kidney Injury and increasing serum creatinine (Piyush.,2022). Serum pro-inflammatory results TNF-α and IL- 6 had significantly changed at 6, 24, 96hrs. and 7thday post-induction of AP as protective mechanism. The body release ant-inflammatory cytokines to regulate the inflammatory response and prevent the over activation of inflammatory reaction. Excessive TNF-α can activate neutrophils and release IL-6 and other cytokines. TNF-α acts as an inhibitor of severe acute pancreatitis complication including liver damage alone can cause toxic shock symptoms. These results agree with (Hotamisligil, 2006; Koizumi et al., 2006 and Carroll et al.,2007). In our study, L-arginine caused a considerable rise in nitric oxide levels at the 6, 24, 96 hrs. and 7th days of acute pancreatitis. Oxidative stress is a condition of imbalance in the body's oxidant and antioxidant activity, with a bias towards oxidation, resulting in neutrophil inflammatory infiltration, increased protease secretions, and the creation of significant quantities of oxidative intermediates NO. These results agree with (Abdel-Aziz et al., 2020). Inducible NOx activation produces apoptosis mediators and these effects may explain the structural changes in the cytoplasm and nucleus detected in induced pancreatitis of the present investigation. In the present work induction of AP was associated with significant increase in serum activities of ALT and AST at 6, 24, 96 hrs. and 7thday groups compared with control one, because AP inducing oxidation stress leading to damage at different organs. Liver is the first extra pancreatic attacked by high concentration of activated digestive enzymes and inflammatory mediators because a part of pancreatic blood flow back through the portal vein. These results agree with (Halit Özgül et al., 2019; Kanwal et al., 2021; Nishikawa, 2000 and Itani et al., 2002).In other hand there was non-significant decrease in cholesterol of AP group in compared with control one at6 and 24hrs.post- induction of AP ,this results may be due to negative relation between lipase and cholesterol when lipase enzyme elevated due to AP induction by one dose of L-arginine at 6 and 24hr. post inoculation reducing cholesterol level, while at 96 hr. cholesterol was recorded significant increase may due to decreased of lipase enzyme approaching to control group. However at 7th day serum cholesterol was recorded significant decreased may be due to one dose of L-arginine injection affected on liver that responsible for the biosynthesis of a large part of cholesterol, this opinion was supported by(Eman and Mohamed 2006) who concluded that improvement in serum cholesterol levels may be due to stimulation of cholesterol excretion into the intestine, stimulation of cholesterol oxidation to bile salts, blocking cholesterol reabsorption from the gastrointestinal tract, preventing bile salt reabsorption, and inhibition of cholesterol synthesis. Present study recorded there was a non-significant rise in blood triglyceride levels in all checkpoints compared to control.

5. CONCLUSIONS

Based on the above data, we concluded that a single L-arginine dosage can induce reversible acute pancreatitis in rats via oxidative and inflammatory effects, with liver and kidney consequences.

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